Individual Differences of Responses to Acute Stress Associated with Type of Behavior. Structural Changes in the Brain

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Typological specificities of animals have not been taken into account in morphological studies devoted to structural alterations in the brain during stress [1,3,5,7]. At the same time, it is a well-known fact that responses to the same stress agent may differ in different individuals and depend on their individual peculiarities.

The aim of the present study was to examine stress-induced structural changes in the brain of rats with different types of behavior which exhibited different degrees of resistance to stress. The data on the resistance of rats with different types of behavior were reported in an earlier publication [9].

MATERIALS AND METHODS

The study was carried out on three groups of rats: 1) rats with an active type of behavior; 2) rats with a passive type of behavior; and 3) an intermediate group. The type of animal behavior was determined by the method described earlier [8]. Each group comprised control and experimental subgroups. Animals twice subjected to behavioral tests and sacrificed directly after the second test were assigned to the "active control." Animals subjected to a single behavioral test (to determine their type of behavior) and sacrificed 1 month later for morphological studies were assigned to the "passive control." The rats of the experimental subgroups were subjected to acute stress before the second test [9]. A total of 30 rats (15 experimental animals, 9 active controls, and 6 passive controls) were used in the study. The brain was prepared for light microscopy after Nissl and Kahal. For electron microscopy, pieces of brain sensorimotor cortex were fixed first in 5% glutaraldehyde and then in a 1% solution of OsO_4 in phosphate buffer, after which they were dehydrated in alcohols and embedded in Araldite. The sections were prepared with the aid of an LKB-III Ultratome, contrasted with lead citrate after Reynolds [12], and examined under a JEM-100-B electron microscope. For quantitation of the density of synapse distribution in 2-3 layers of the sensorimotor cortex, the brain was treated with phosphotungstic acid, which stains only active zones of the synapses. Active zones were counted on the monitor of the electron microscope (×20,000) in 100 fields of 13.5 μ^2 each. Statistical processing was performed using Student's t test.

RESULTS

Regardless of the type of behavior, no significant changes were revealed in the neurons, glia, syn-

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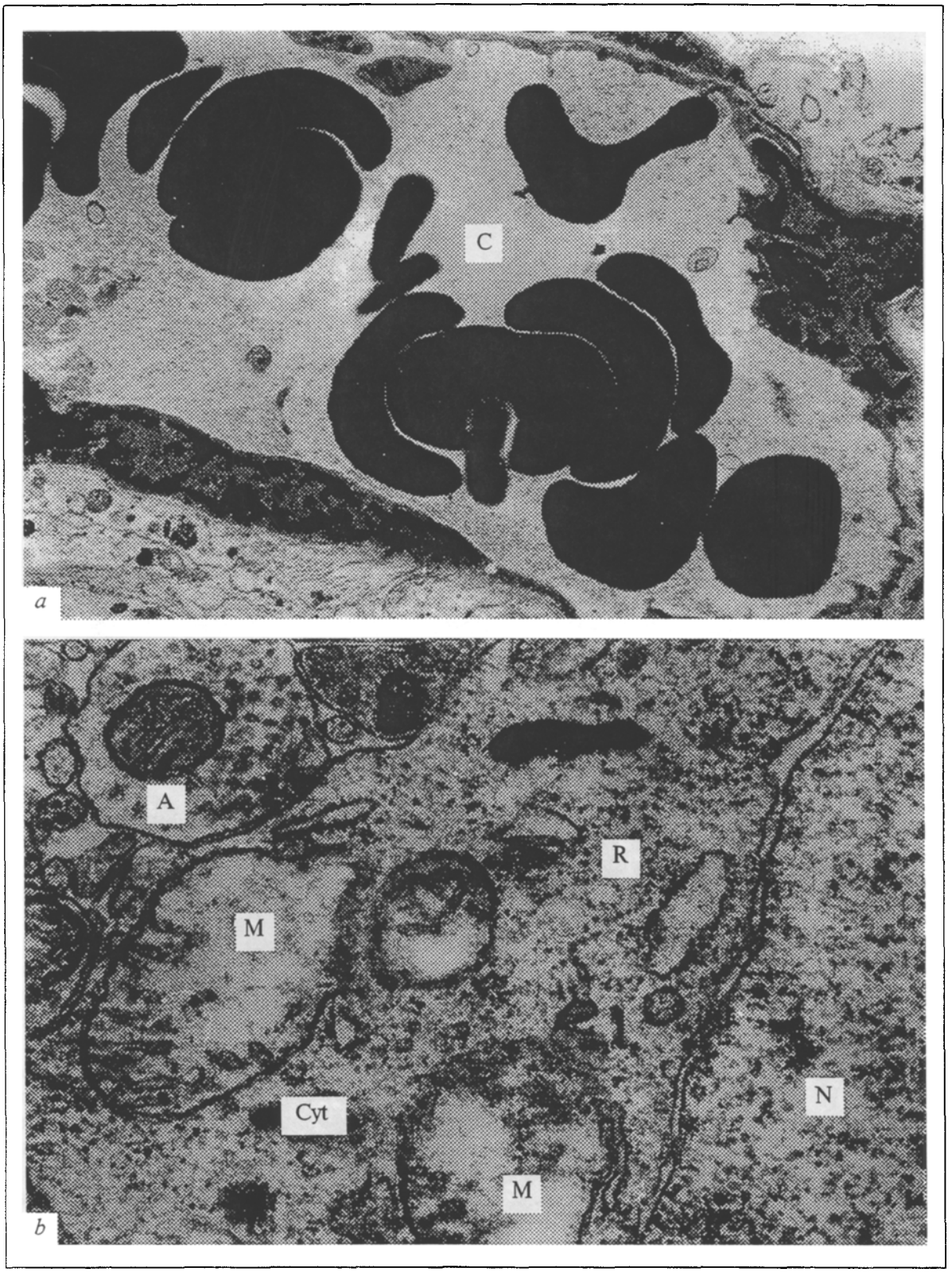

Fig. 1. *a*) marked enlargement of lumen of capillary in brain sensorimotor cortex in a rat with the passive type of behavior one hour after stress. Accumulation of erythrocytes in the lumen. C: lumen of capillary. ×10,000. b) swelling of mitochondria, dissociation of cristae of mitochondria and of granular reticulum in cytoplasm of neurocyte in sensorimotor cortex in a rat with the passive type of behavior one hour after stress. Arrow: axon terminal forms axo–somatic synapse. A: axon terminal; R: ribosome; Cyt: cytoplasm; N: nucleus. ×56,000.

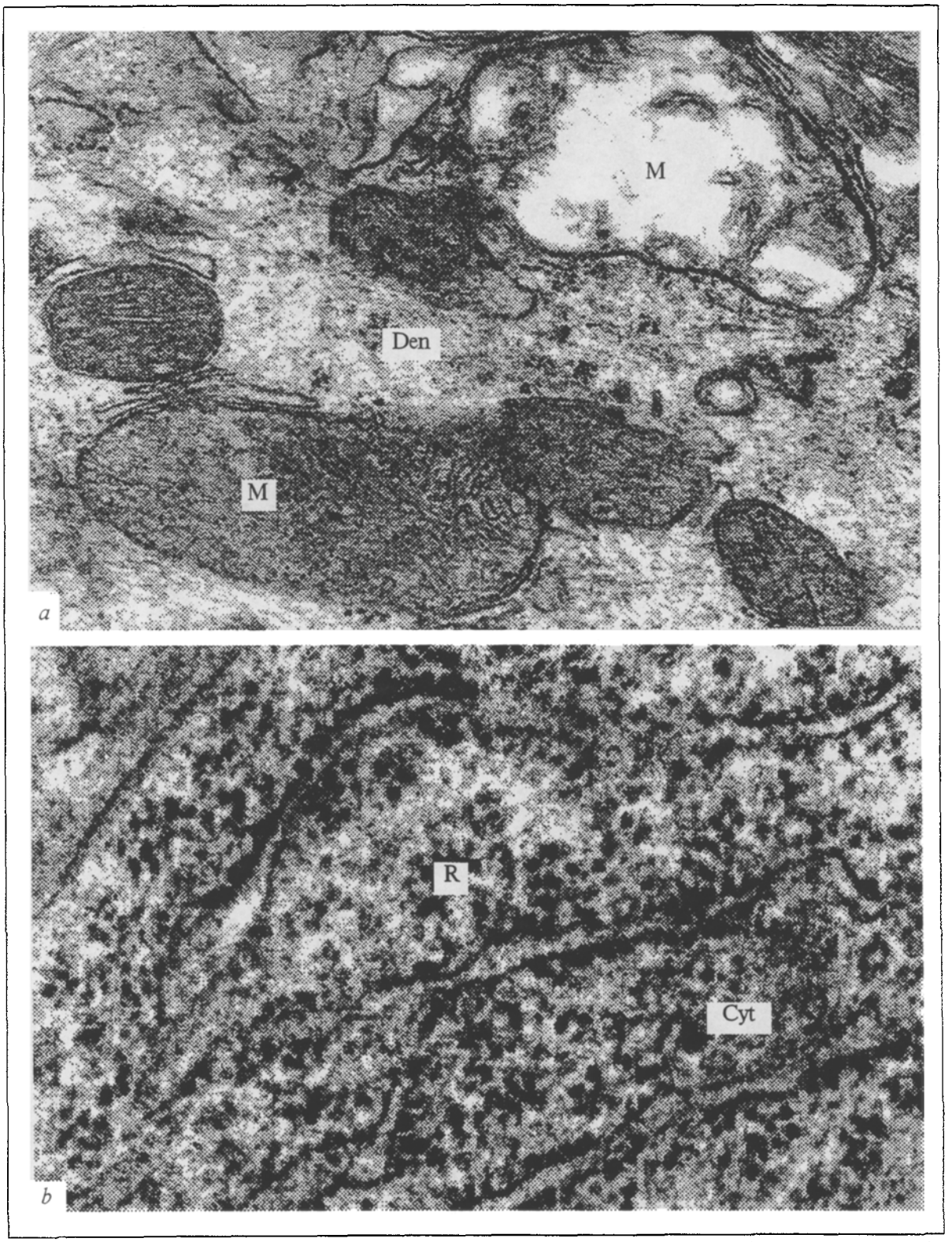

Fig. 2. *a*) combination of destructive (dissociation of cristae) and reactive (hypertrophy) changes in mitochondria in dendrite of neurocyte of sensorimotor cortex in a rat with the active type of behavior one hour after stress. Den: dendrite; M: mitochondrion. ×60,000. *b*) increased number of ribosomes in neurocyte cytoplasm in a rat with the active type of behavior one hour after stress. R: ribosome (polysome); Cyt: cytoplasm. ×100,000.

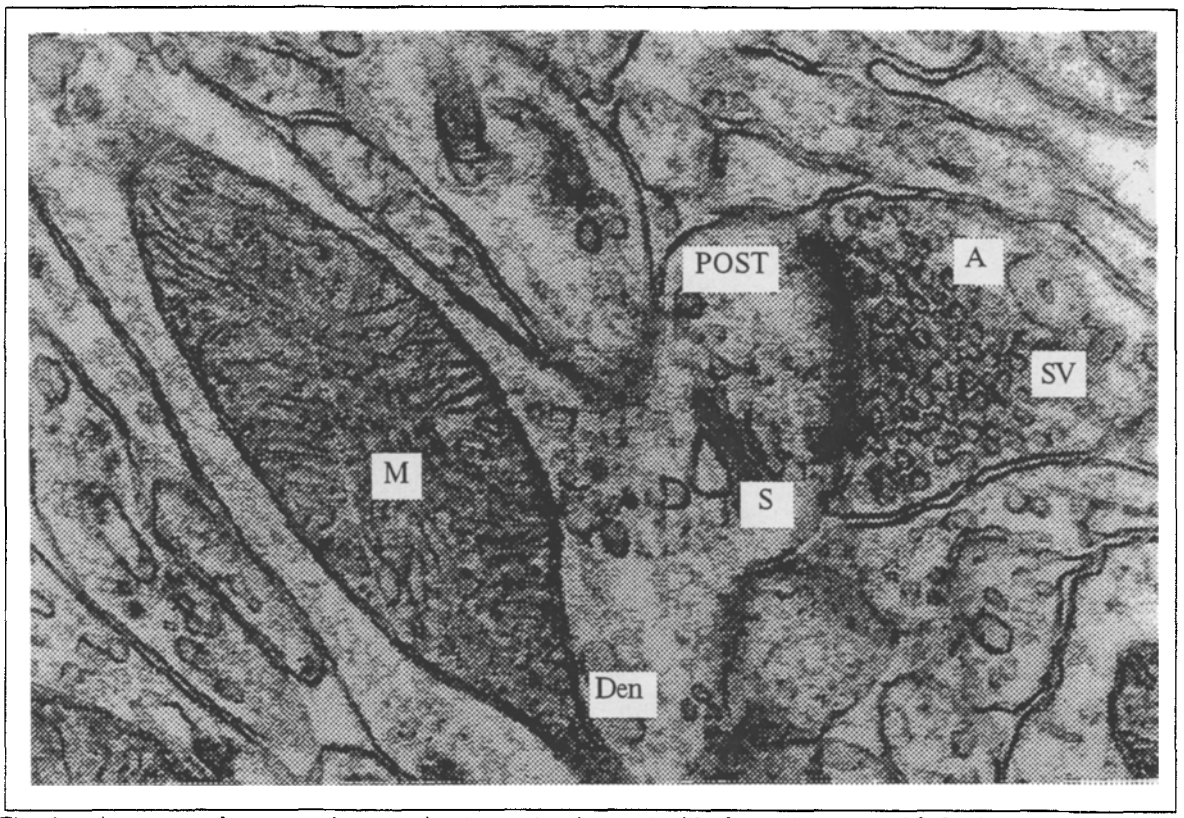

Fig. 3. Axo-spunal synapse in sensorimotor cortex in a rat with the active type of behavior one hour after stress. Hypertrophied mitochondrion seen at base of dendrite spine. Cluster of synaptic vesicles seen in axon terminal. Postsynaptic membrane markedly thickened. A: axon terminal; Den: dendrite, M: mitochondrion; SV: synaptic vesicles: POST: postsynaptic membrane; S: spine. ×60,000.

apses, and blood vessels of the brain in the active as well as in the passive controls.

One hour after acute stress, strongly marked hyperemia of the vessels, especially of capillaries, enlargement of the lumens, and accumulation of the formed elements of the blood were observed in the rats of all experimental subgroups, irrespective of the type of behavior (Fig. 1, *a*). The most typical response of the brain tissue to stress was the changed structure of the granular reticulum in the cytoplasm of the nerve cells: the count of ribosome elements (both free and fixed on the membranes of the reticulum) was reduced. The degree of dissociation of ribosome elements varied in different nerve cells. In some of them the dissociation was focal, while in others it assumed a diffuse character, i.e., it spread all over the cell cytoplasm. Dissociation of the granular reticulum was accompanied by deformation of the channels in the reticulum, and was frequently attended by degeneration of mitochondria, not only the inner structure of the mitochondria but also the outer membrane being involved in this process. Destruction of cristae was observed, both with and without swelling of mitochondria. The above-mentioned destructive processes were encountered in the brain of rats of all three groups; however, these processes were most pronounced in the rats with the passive type of behavior (Fig. 1, *b*).

Another typical response of the brain to acute stress consisted of reactive changes manifested in hypertrophy and division of mitochondria. These processes were variously pronounced in different rat groups and in different animals within one and the same group. Nevertheless, on the whole they were more marked in rats with the active type of behavior (Fig. 2, *a*). In individual cells, especially in rats with active behavior, hyperplasia of the protein-synthesizing organoid was found (Fig. 2, *b*). In the rats of the intermediate group both reactive and destructive changes were observed in the brain, these changes being highly variable in individuals within this group, as well as in different cells in one and the same animal. A combination of reactive and destructive processes in the brain of dogs was previously described for experimental neurosis [5].

The wide polymorphism of the changes in the protein-synthesizing system of different brain cells in the same animal attested to a selective (local) change of synapse activity, leading to a change of protein metabolism just in the neuronal populations concerned. In other words, ultrastructural changes

in brain neurons directly depend on the topography of synaptic complexes which are involved in the stress response and which are evidently associated with the specificity of the stress factor. However, one hour after stress the number of synapses per unit area of sensorimotor cortex did not change reliably in the rats of all three groups. For instance, it constituted 2.34±0.12 in the rats with active behavior (vs. 2.24±0.11 in the control), 2.19±0.13 in the rats with passive behavior (vs. 2.12±0.12 in the control), and 2.09±0.11 in the rats of the intermediate group (vs. 2.29±0.12 in the control). Since changes in ribosome elements, which were the first to respond to the changes in the functional state of the synapses [2,4,11], occurred in the cells, one may assume that ultrastructural changes in the brain caused by acute stress stem from enhanced synaptic activity of the synaptic complexes at hand. The synapses we discovered with a high content of synaptic vesicles, with markedly thickened and elongated synaptic membranes in the active zones of the synapses, and with hypertrophied mitochondria near the synapses may morphologically corroborate this conclusion (Fig. 3).

Thus, in the early stage after stress, both reactive (hypertrophy and division of mitochondria and hyperplasia of ribosome apparatus) and destructive (dissociation of the inner structure of mitochondria and granular reticulum) changes appear in the rat brain. Reactive processes are more pronounced in the brain of rats with an active type of behavior, which exhibit the strongest resistance to acute stress [9], whereas in rats with a passive type of behavior, which are less resistant to stress, destructive processes are more marked. The rats of the intermediate group, exhibiting the lowest resistance to stress, demonstrate "indefinite" structural changes in the brain, without a clear predominance of either type.

The reactive changes revealed in the brain are not characteristic exclusively of stress. For instance, changes in the mitochondria and granular reticulum were discovered by us in the brain during circulatory hypoxia caused by bilateral occlusion of the carotid arteries [8], and changes were observed by other authorities in the muscle tissue during intensive physical loads [10]. At the same time, in ischemia of the brain, early reactive changes in the mitochondria prevail over all other changes, and hypertrophy precedes the destruction of mitochondria and granular reticulum. During acute stress, hypertrophy and division of mitochondria are far less pronounced; they have a focal localization and go along with destruction of mitochondria and of the protein-synthesizing organoid. Mitochondrial hypertrophy, directed toward the enhancement of aerobic power in the tissue and preventing a rise of the lactate level in the brain (one of the leading factors in destructive processes), is, essentially, a nonspecific (antihypoxic) adaptive response. Possibly, individual differences in the resistance not only to circulatory hypoxia of the brain, as found previously [8], but also to acute stress, as stated in the present study, are associated with individual differences of reactive changes in the brain, which are a structural manifestation of the mechanism of timely adaptation [6].

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